THE USE OF BASIC PROTEINS TO INCREASE THE INFECTIVITY OF ENTEROVIRUS RIBONUCLEIC ACID

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In the past several years infectivity of the RNA extracted from certain plant and animal viruses has been demonstrated. The infectivity of these RNA preparations is frequently very low compared to that of the whole virus suspensions from which they are derived. Koch, et al., (1960) found that the infectivity of poliovirus RNA could be increased considerably by the use of hypertonic salt solutions (1 M) at an alkaline pH. A similar finding, brought about by the addition of high ionic-strength magnesium sulfate solution (2 M), has been reported by Holland, et al., (1960). In studies with RNA extracted from Escherichia coli, Amos, (1961) found that, by the addition of protamine, he could enhance the uptake of the RNA by cultured chick cells.

Recently a series of experiments was undertaken in this laboratory to study the enhancement of enterovirus RNA infectivity under isotonic rather than hypertonic conditions. It was found that, at a sodium chloride concentration of .14 M and at pH 7, the normally low infectivity of type 1 poliovirus RNA could be markedly increased by the addition of histone* to the preparation. Without histone, such preparations produce only an occasional plaque when adsorbed on Hela cell monolayers for 40 minutes at 36 C. When histone was added prior to adsorption, yields of over 10,000 plaque-forming units per ml were attained.

The RNA preparations were made from crude tissue culture fluid virus suspensions by a modification of the phenol method of Gierer and Schramm (1956).

*Calf thymus nuclei (Nutritional Biochemicals Corporation)

Dilution of the RNA was made in 0.14 M sodium chloride solution and the pH adjusted to 7 with sodium bicarbonate. Prior to inoculation of the monolayers, various concentrations of histone were added to individual portions of the RNA extract. The histone had previously been dissolved in 0.14 M sodium chloride solution. Physiological saline was also used to wash the monolayers prior to their inoculation with the histone-RNA preparations.

Table I shows that histone exerts its maximal effect on increased infectivity when used in a final concentration of approximately 400 micrograms per ml. Furthermore, histone does not cause the cell damage and shedding of monolayers which frequently occur with the use of hypertonic salt solutions.

TABLE I

THE EFFECT OF INCREASING CONCENTRATIONS OF HISTONE ON THE PLAQUE COUNT OF POLIOVIRUS RNA* AT 36 C

Histone Concentration (Micrograms per ml)	Average Number of Plaques per Bottle
50	1
100	3
200	11
400	46
600	25
800	12

*RNA preparation diluted 1:10 in .14 M NaCl containing appropriate amount of histone. Inoculum was 0.15 ml per plaque bottle.

Preliminary studies show a further increase in the infectivity of the histone-RNA preparations when adsorption takes place at temperatures below 36 C. At 20 C and at 4 C the number of plaque-forming units was increased approximately four-fold over the number following adsorption at 36 C.

When histone was used with hypertonic salt solutions at an alkaline pH, the infectivity of the RNA preparations was not increased beyond that brought about by either the histone alone or by the hypertonic salt and alkaline pH system alone.

Further studies showed that the plaque count decreased linearly with dilution of the histone-RNA preparations. Ribonuclease in a final concentra-

tion of 10 micrograms per ml was found to inactivate rapidly these same preparations.

Under similar conditions protamine sulfate was also found to increase the infectivity of poliovirus RNA.

Since both histone and protamine are rich in arginine, the effect of this amino acid on the infectivity of the RNA was tested. Although concentrations ranging from 200-2400 micrograms per ml were used, arginine was without effect.

The infectivity of Coxsackie B_3 virus RNA, in isotonic sodium chloride solution at pH 7, was also increased by addition of histone.

A more complete report on these findings will follow. The effects of variations of such factors as pH, temperature, and length of adsorption period on the basic protein-RNA system are being studied at the present time. Other basic proteins, polyamines, diamines, and certain combinations of these substances are under investigation for their ability to enhance the infectivity of enterovirus RNA under isotonic conditions.

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